VOLUME 15 NUMBER 8

NUTRITION REVIEWS

August 1957

Nutrition in the Mammalian Embryo by O. A. Schjeide 225

Clinical Nutrition

- Dietary Protein and Serum Cholesterol 227
- Hypernatremia 229
- Fat and Nitrogen Absorption in Gastrectomized Patients 230
- Renal Handling of Potassium 231
- Experimental Magnesium Deficiency in Man 232
- Idiopathic Hypoproteinemia 232
- Protein Malnutrition in Brazil 234
- Body Water 236
- Magnesium and Electrolyte Excretion 237

Animal Nutrition

- Dietary Enterococci and Growth of Chicks 239
- The Nutritive Evaluation of Forages 240

Experimental Nutrition

- Dietary Protein and Experimental Tuberculosis 243
- Ascorbic Acid Requirement with Periodic Administration 244
- Potassium and Nitrogen Balance 245
- Utilization of Urea by the Rat 246
- Minimal Resection of the Small Intestine and Absorption of Vitamin A 248
- Bone Erosion During Lactation 249
- Vitamin D and Cortisone—Synergists or Antagonists? 251
- Vitamin D and Citrate Oxidation 253

Notes

Letter to the Editor 254

- Total Exchangeable Sodium in Hypertension 255

Recent Nutrition Books 256

Erratum 256

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NUTRITION REVIEWS

VOL. 15 AUGUST 1957 No. 8

NUTRITION IN THE MAMMALIAN EMBRYO

Biologists will generally agree that intimate acquaintance with the morphology of the embryo is requisite for a proper concept of adult anatomy. With similar logic it has been proposed that metabolic mechanisms of the adult may be more clearly understood by observation of their elaboration during embryonic and fetal life. The practical difference between these two notions is that whereas it is fairly easy to trace morphological changes throughout development, to describe biochemical etiology with any accuracy is relatively difficult.

Yet, if the nutrition of living things is to be considered from a most general point of view (which is, of course, the most fundamental approach) studies of metabolism of foodstuffs in the developing organism have their place. Armed with little more than ingenuity, skill, and hope, the nutritional embryologist assails an implacable fortress, but in so doing avails himself of the possibility of unveiling most unique and useful information.

Of the many barriers to investigation of nutrition in the mammalian embryo, one of the more formidable is the maternal organism. Obviously, the embryo has access only to those nutrients passed to it through the circulation of the mother. Of the total incoming foodstuffs only a fraction will be distributed to each compartment. Certain incoming substances, because of maternal utilization or conversion, may never reach the embryo. On the other hand, the maternal organism is capable of synthesizing or mobilizing a number of materials which may be essential or conducive to an optimal growth environment. To a considerable, but probably variable, degree, the nutrition of the embryo is a function of the food intake of the mother. However, the embryo takes priority over the mother with regard to certain foodstuffs.

Quite probably, nutrients pass from the circulation of the mother to that of the embryo by simple diffusion rather than by active "pumping" or secretion. Data on Ca^{45} transfer by C. L. Comar (*Ann. N. Y. Acad. Sci.* **64,** *281* (*1956*)) show that the calcium level of the fetal circulation is distinctly lower than that of the maternal circulation. It would appear that the major reason for rapid deposition of calcium in the fetal skeleton is the extreme avidity of the bone tissue for calcium. This view is borne out by a recent comparison made of calcium accretion in growing boys and adult men (F. Bronner and R. S. Harris, *Ann. N. Y. Acad. Sci.* **64,** *314* (*1956*)). Although the same amount of radio-calcium was injected into individuals of both age groups, the retention was several times greater in the boys. It is such retentiveness by the tissues of the growing organism which largely favors the fetus in the maternal-fetal relationship. However, it is possible that, in addition to the direct demand of growing tissues, mechanisms generated more or less exclusively by the dam operate in favor of the fetus.

The site of transfer of nutrients from the mother to the fetus is not always limited to the placenta. In the rabbit and certain other species which exhibit a yolk sac-type of splanchnopleure, there is evidence of diffusion of nutrients from the uterine circulation into the horns of the uterus and thence into the circulation of the fetus via the fetal membranes (F. W. R. Brambel, W. A. Hemmings, M. Henderson and C. L. Oakley, *Proc. Roy. Soc.* **B., 139,** *567* (*1952*)). It is not yet known whether or not those mammals which possess an allantoic-type placenta (the human is included in this group) ordinarily absorb a significant proportion of nutrients from the uterine cavity. The external membranes of the embryos of these species are not so extensively vascularized

as are those of animals having yolk-sac type placentas.

Another of the problems facing the nutritional embryologist resides in the fact that embryos and fetuses themselves present certain characteristics not found to the same degree in adult organisms. First, they are rapidly growing. Such growth involves at least two uniquely distinct mechanisms, cellular division and increase in cellular volume. Secondly, they are rapidly differentiating. Differentiation takes place both at the intra- and extra-cellular levels. Thus, there is a considerable difference in complexity and probably, also, in total and partial sensitivity to nutritional deficiencies as a function of age. Of course, these are precisely the factors which provoke investigation; the difficulty is that there are so many of them and that they are so poorly understood!

Fortunately for those who are interested in assessing the relative influences of such factors, development of the mammalian organism can be divided into three fairly different metabolic and morphological phases: (1) the pre-implantation period; (2) the interval during which major organogenesis takes place; (3) the period of most rapid prenatal growth. In the human, the pre-implantation period extends for approximately two weeks after conception; the duration of major organogenesis is from two weeks to eight weeks; and the period of prenatal growth extends from the eighth week to term.

If the studies of N. T. Spratt (*Biol. Bulletin* **99,** *120* (*1950*)) and the Edinburgh school on the chicken embryo can be applied to the mammal, it would appear that carbohydrates may be preferentially utilized during the first interval of growth, whereas amino acids are especially required during organogenesis, and lipids and proteins are utilized to the greatest extent during the period of major growth. Unfortunately, most of the information on the latter stages is from respiration data. Considerable overlapping in utilization of such general categories of foodstuffs must take place although the investigations of M. D. Francis and T. Winnick *J. Biol. Chem.* **202,** *273* (*1953*) clearly indicate that whole proteins are not utilized by chicken heart cells until about the fourth day of incubation.

In many studies of embryo and fetal nutrition it has been inherently assumed that only those molecules which are small enough to passively dialyze through animal membranes will reach the embryo. However, it has recently been demonstrated (P. F. Knight and A. M. Schechtman, *J. Exp. Zool.* **127,** *271* (*1954*)) that foreign proteins injected into the circulation of the hen will appear in serologically detectable form in the egg-yolk. In this laboratory evidence has been obtained (O. A. Schjeide and M. Urist, *Science* **124,** *1242* (*1956*)) that phosphoprotein (vitellin) of the egg has probably been transferred intact from the circulation of the hen to the yolk. Injection of foreign proteins into the uterine lumen (as well as into the circulation) of the pregnant rabbit has also resulted in the detection of these moieties in the fetal circulation (Brambel, *et al., loc. cit.;* Hemmings, *Proc. Roy. Soc.* **145,** *186* (*1956*)); A. C. Kulangara, *Personal Communication*). There may be considerable differences in the rate of uptake of macromolecules as a function of the age of the embryo. In the case of the rabbit, the older fetus assimilates considerably more protein through the external membranes than do younger stages. Studies conducted with foreign proteins are now being checked in this laboratory with homologous C^{14}-labeled proteins (Kulangara). In a previous significant work with C^{14}-labeled proteins, M. D. Francis and T. Winnick (*J. Biol. Chem.* **202,** *273* (*1953*)) demonstrated that embryonic heart cells of the chicken are permeable to, and can utilize for structural protein synthesis, whole proteins, if these are present in tissue culture media. When this result is compounded with our finding that the two-component protein system "vitellin"

is present in soluble form in the circulation of the hen (Schjeide and Urist, *loc. cit.*), and in the form of granules in the egg yolk, it appears fairly certain that some structural proteins within embryo cells are derived intact from the mother.

If a comprehensive evaluation of the nutrition of the mammalian embryo is to be attempted, it is also necessary to know what *type of response* will be elicited when a given deficiency is imposed at a given point in development. Since nutritional insults are difficult to apply at precise time intervals, the problem is again formidable. Unexpected assistance has come, however, from studies of developmental radiobiology (L. B. Russell, *Radiation Biology* **I**, *Part 2*, *861* (*1954*)). It has been found that when radiation is applied during the pre-implantation period, there is diminution in litter size as a function of radiation dose but in the animals born, *no abnormalities* are observed. With doses of radiation above a certain level it is known that death of some cells of the blastula must occur. However, the cells at this stage are apparently omnipotent so that those which are not destroyed can take over to produce a complete organism.

When irradiation is administered during the period of rapid organogenesis, it is found that the incidence of prenatal death is considerably lower than when the radiation is given in the earlier stage but the number of abnormal offspring increases markedly. Nearly one hundred per cent incidence of abnormalities occurs with doses greater than 100 roentgens. These include eye, brain, and nerve defects, skeletal and urological derangements, cleft palate, etc. The reproducibility of a given abnormality is sharply delineated by the exact time of irradiation.

Irradiation administered during the major period of prenatal growth has little effect on either growth or viability unless the dose is elevated to approximately that which would be effective in the adult organism. No gross abnormalities are induced.

From further experience with a number of different agents it seems likely that the embryo should respond to radiation and nutritional insults in a similar fashion, the response being more dependent on the time at which the insult is applied than on the exact nature of the insult. Thus, T. H. Ingalls, (*New Eng. J. Med.* **243,** *67* (*1950*)) has made out an excellent case for mongolism being caused by nutritional factors (and others) operating at about the eighth week of fetal life. Likewise it has been shown by A. Giroud, H. Gounelle and M. Martinet (*Bull. Soc. Chem. Biol.* **39,** (*1957*)) that excessive doses of vitamin A are most effective in producing abnormal rat fetus when they are administered to the mother during the 12th, 13th and 14th day of gestation, that is to say, during the period of major organogenisis.

Other experiments have been carried out which show that specific and general nutritional deficiencies produce abnormalities similar to those listed above for radiations (J. Warkany, *J. Pediat.* **25,** *476* (*1944*)). A variety of species has been tested with analogues of amino acids, folic acid analogues, nicotinic acid analogues, and thiamine analogues. W. W. Cravens (*Ann. N. Y. Acad. Sci.* **55,***188* (*1952*)) has reviewed this work and lists the types of abnormalities observed. Unfortunately, it is impossible from most of the work done thus far to sort out the numerous effects and establish the details of their occurrence. New investigations should be planned with attention being given to (1) the relationship of specific nutritional deficiencies to developmental age, (2) the special physiology of that stage, and (3) the exact nature of the response. Utilization of radio-labeled nutrients should prove a valuable adjunct to such studies.

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DIETARY PROTEIN AND SERUM CHOLESTEROL

The results of various national surveys have shown that human populations subsisting on diets low in fat tend to have low concentrations of serum cholesterol (*Nutrition Reviews* **11,** *292 (1953); Ibid* **14,** *67 (1956)*). Since, in many of the areas that have been surveyed, diets that were low in fat were also known to be low in total protein, or at least in animal protein, A. Keys and J. T. Anderson (*Am. J. Clin. Nutrition* **5,** *29 (1957)*) conducted a series of experiments on human subjects in order to assess the influence of two different levels of protein intake on serum cholesterol values.

The subjects were physically healthy, schizophrenic men, of 32 to 54 years old. They were maintained under carefully controlled conditions in the metabolic unit of a hospital. In the first experiment, 17 men were maintained for four weeks on a nutritionally complete diet which provided about 3000 Calories, 85 g. of protein, and 55 g. of fat. They were then divided into two groups which were matched on the basis of a wide variety of factors. For a further four weeks the diet of one group of nine men was the same as the control diet, except that butterfat was substituted isocalorically for carbohydrate to provide 125 g. of fat (almost 40 per cent of the Calories); the other group of eight men received a diet with the same increase in fat content, but in which the protein was also increased from 85 to 130 g. by substituting skim milk powder for another part of the carbohydrate.

In a second experiment, in which there were 25 subjects, the design was such that each man served as his own control. In this experiment the fat content of the diet was kept constant throughout at approximately 20 per cent of the caloric intake, and the basic protein intake was decreased to a little below 70 g. per day. The four periods of four weeks each consisted of a control period, a period during which 1 g. of cholesterol per day was added, a period during which cholesterol was added and the protein intake was approximately doubled, and finally, another control period. The subjects were divided into two groups, for one of which the sequence of periods two and three was reversed.

Total serum cholesterol and the cholesterol in the alpha lipoprotein fraction were estimated directly during the last few days of each four-week experimental period. The beta-lipoprotein cholesterol was computed by subtracting the value for the alpha fraction from the total.

The only dietary treatment which significantly altered the serum cholesterol values in any of these experiments was the increase in fat intake in the first experiment. The control values for the subjects receiving the moderate protein level and the high protein level were 193 $\pm$ 12 mg., and 213 $\pm$ 14 mg., of cholesterol per 100 ml. of serum, respectively. After they had consumed about 125 g. instead of 50 g. of fat per day, the values increased 23.0 $\pm$ 5.6 mg. and 25.8 $\pm$ 7.5 mg. per 100 ml. of serum, respectively.

In the second experiment no effect on serum cholesterol level was detected when the daily protein intake was raised from between 65 g. and 70 g. per day to between 125 g. and 140 g. per day. Even the ingestion of 1 g. of extra cholesterol daily did not cause a significant increase in the serum cholesterol value (*Nutrition Reviews* **14,** *67 (1956)*).

The experiments appear to be a model for studies on human subjects. However, the authors might have given some details about the composition of the basic diet. Such information as the type of fat in the basic diet would be of interest in view of the differences that have been observed in the effects of various dietary fats on serum cholesterol (*Nutrition Reviews* **15,** *1 and 39 (1957)*).

The four-week experimental period used by Keys and Anderson is short in terms of

the human life span, but it was sufficient to demonstrate an increase in the level of serum cholesterol when 75 g. of butterfat was added to the daily ration. Although the results do not provide any support for the hypothesis that the low serum cholesterol values of populations consuming diets low in fat are related to the amount or kind of protein in their diets, the authors recognize the danger of extrapolating beyond the relatively short time periods of their experiments.

HYPERNATREMIA

The human infant has relatively great requirements for water, amounting to three times that of the adult per unit of body weight. During the course of the development of diarrhea in small infants, water losses tend to be larger than electrolyte losses (*Nutrition Reviews* **10,** *293* (*1952*)) especially in hot weather, and the ability of the kidney during the first year of life to handle loads of sodium may be reduced (*Nutrition Reviews* **11,** *169* (*1953*)).

In small infants the occurrences of diarrhea, low water intake, and high water losses frequently lead to the accumulation of sodium in the extracellular fluid. Hypernatremia is defined by L. Finberg and H. E. Harrison (*Pediatrics* **16,** *1* (*1955*)) as a condition in which the concentration of sodium in the serum is greater than 150 mEq per liter. In 88 infants with hypernatremia that Finberg and Harrison studied, the mortality was 11.6 per cent as against 2 per cent in infantile diarrhea not complicated by hypernatremia.

Although these infants are dehydrated, they do not appear so since the high sodium content of the extracellular fluid maintains its volume at the expense of the cellular water. The skin has a sclerema-like texture. The more prominent symptoms involve the central nervous system, with alterations in consciousness, irritability, muscular twitchings or convulsions. The spinal fluid protein concentration was increased in 30 of 32 patients so examined. Neurologic residua occurred in four of 69 patients with central nervous system involvement. Finberg and Harrison point out that the water losses in the stools of these patients with non-specific diarrhea are in excess of the losses of sodium or other electrolytes, and that the negative water balance is further aggravated by diminished water intake and by the presence of hyperventilation due to fever.

W. B. Weil and W. M. Wallace (*Pediatrics* **17,** *171* (*1956*)) found hypernatremia in 20 per cent of infants admitted with diarrhea. They had the opportunity of studying the urine in a few of their patients before treatment was started. The urine was dilute and a very low urea clearance was found, suggesting considerable renal insufficiency. Balance studies on five patients showed, on recovery, a gain in water of between 40 and 80 g. per kilogram, very little change in the sodium and potassium balance, and a loss of chloride of approximately 6 or 7 mEq per kilogram. Interpretation of these studies suggested that the water loss was largely intracellular.

A. L. Skinner and F. C. Moll (*Am. J. Dis. Child.* **92,** *562* (*1956*)) found hypernatremia accompanying infant diarrhea in 25 per cent of their cases. Most of the patients reported by Skinner and Moll had received moderate to large amounts of sodium by mouth or parenterally before they were seen, and often relatively large amounts of sugar solutions containing 1 teaspoonful of common table salt per quart of water. These authors point out that such solutions given orally may exceed the ability of the relatively immature kidneys of the small infant to excrete sodium. On the basis of their experience, Skinner and Moll feel that the ceiling for renal excretion of sodium may be

in the range of 5 to 10 mEq per kilogram of body weight per day.

All three groups of workers have similar suggestions for the treatment of these infants consisting of moderate amounts of fluid containing sodium chloride and other electrolytes in quite low concentration. The administration of non-electrolyte containing solutions in the treatment of these infants is often followed by convulsive episodes simulating acute water intoxication.

The relatively frequent occurrence of hypernatremia in infantile diarrhea, especially when aggravated by the feeding of relatively dilute solutions of sodium chloride by mouth, would suggest that the ability of the infantile kidney to excrete sodium loads is greatly reduced in this diseased state. Under the above circumstances, the excessive administration of salt to infants will certainly produce a profound metabolic disturbance. On the other hand, clinical situations such as Addison's disease occur in which the oral administration of large amounts of salt over a long period of time may be a lifesaving measure. Consequently, it must be pointed out that the ability of the young human organism to excrete amounts of salt easily ingested varies between wide extremes, and that intakes of sodium chloride, which may be beneficial under some circumstances, may be toxic in others.

FAT AND NITROGEN ABSORPTION IN GASTRECTOMIZED PATIENTS

Perfected surgical techniques have permitted the extended use of total gastrectomy in gastric cancer. Observation of the patients after surgery has revealed a number of metabolic defects, among which malabsorption of fat and nitrogen is prominent. It has been demonstrated both in animals (*Nutrition Reviews* **13,** *86 (1956)*) and in man (*Ibid.* **14,** *266 (1956)*) that gastrectomy materially increases fecal fat and nitrogen, adequately accounting for the negative caloric balance. M. K. Schwartz, O. Bodansky, and H. T. Randall (*Surgery* **40,** *671 (1956)*) examined the extent to which a variety of drugs and procedures can influence fat and nitrogen absorption in gastrectomized patients.

Nine patients were studied by classical balance techniques. Pancreatin powder, of 18 g. per day, did not affect the nitrogen absorption. The steatorrhea exhibited by all patients was not improved and, in fact, it possibly was increased. In a further attempt to increase fat absorption, 18 g. of a purified lipase was given in divided doses to three patients. In one patient, the per cent of the fat intake excreted dropped from 29 to 18. However, the lipase caused a severe diarrhea in two other patients, with a consequent increase in fecal fat and nitrogen.

If the steatorrhea seen in the gastrectomized patient were due to decreased stimulation of pancreatic secretion, inadequate mixing of the food with these enzymes, or a deficiency of pancreatic enzymes, then the administration of pancreatic enzymes would be expected to increase fat absorption. This was not observed with pancreatin. The authors concluded that the lipase was ineffective, although it would seem that one positive result is of greater significance than the two negative studies which were complicated by diarrhea.

To test the possible role of decreased emulsification as a cause of steatorrhea, Sorlate®, an emulsifier, was given to two patients. Given in a dose of 6.0 g. per day, no effect was noted.

It has been suggested that increased intestinal motility, noted after gastrectomy, may adversely affect absorption of fat and nitrogen. A ganglionic blocking agent and, subsequently, a partial sympathectomy failed to affect fat absorption in one patient. In an attempt to simulate the function of the stomach in introducing small amounts of

foods into the intestine at frequent intervals, the effects of frequent feedings were studied. One patient had increased fat absorption when eight feedings were used instead of two, but the other patient's fat absorption was unchanged by the same maneuver.

Although these studies have not provided definitive answers to the problem of the malabsorption of fat and nitrogen after total gastrectomy, they contribute materially to our knowledge of this disorder. The single result with lipase needs verification. The role of the stomach remains obscure, and its suggested action in triturating the food and controlling the flow of chyme is but one of a number of hypotheses which remain to be tested.

RENAL HANDLING OF POTASSIUM

Current concepts regarding the excretion of potassium by the kidney are that the potassium filtered at the glomerulus is reabsorbed, and that urinary potassium is secreted by the renal tubules. Considerations of the exchange rates of radioactive potassium (K^{42}) have permitted greater support for this hypothesis.

D. A. K. Black, H. E. F. Davies, and E. W. Emery (*Lancet* **I,** *1097* (*1955*)) were able to show that K^{42} given intravenously was very quickly distributed in tissues, somewhat in proportion to blood flow rather than tissue potassium content. For example, the kidneys receive about 20 per cent of the cardiac output, but contain only 1 to 2 per cent of the body potassium. Two minutes after injection of K^{42} in rabbits, each kidney may be shown to contain up to 10 per cent of the injected dose. Thirty minutes after injections, the kidneys contained only 2 per cent of the dose, a value which agrees well with the tissue content of potassium.

The implications of this type of study were discussed by the authors, who stated that in any capillary bed the amount of potassium exchanged will depend on the speed of perfusion, as well as the speed of exchange. If the transit time is much shorter than the exchange time, then most of the entering K^{42} will leave in the venous blood and the tissues will take up K^{42} in proportion to their exchangeable potassium. If, however, the exchange time is much shorter than the transit time, perfusing K^{42} will rapidly enter the tissues, the plasma K^{42} levels will decrease rapidly, and the initial distribution of K^{42} will be in proportion to blood flow. The experiments cited favor this latter interpretation.

The renal handling of potassium in humans was then examined by D. A. K. Black *et al.* (*Clin. Sci.* **15,** *277* (*1956*)). They continuously sampled brachial artery blood, renal venous blood obtained by catheter, and urine after intravenous injection of 80 microcuries of K^{42}. In three subjects studied, the K^{42} appearing in renal vein blood in the first 30 seconds averaged only 6 per cent of the arterial input. Even this figure may be high, as some of the potassium may have entered the renal tissue and left it again. Such high exchange rates have been demonstrated with other isotopes.

During the next half-hour, the specific activity (% dose/mEq potassium) of the potassium in the renal vein was higher than in the renal artery. This follows from the previous observations suggesting the initial high rate of exchange of potassium in the kidney, thus leaving the kidney labeled with potassium of high specific activity.

For an hour or more after the injection of the radioactive potassium, the specific activity of the urine potassium was higher than arterial potassium, but it closely approximated the specific activity of the potassium in the renal vein. This is explained by the hypothesis that urine potassium and renal vein potassium have a common source in renal tissue, whereas arterial potassium

makes little contribution to urinary potassium via glomerular filtration. As the assumptions made by the authors regarding blood flow and sampling procedures seem eminently reasonable, these experiments are good evidence that urinary potassium originates from renal tubular secretion of potassium.

EXPERIMENTAL MAGNESIUM DEFICIENCY IN MAN

Although hypomagnesemia and magnesium depletion have been noted in several clinical settings, no experimental study of pure magnesium depletion in man has been reported. M. G. Fitzgerald and P. Fourman (*Clin. Sci.* **15**, *635 (1956)*) undertook to examine this problem and were able to study the development of magnesium deficiency in two normal males.

As most food contains important amounts of magnesium, the diet was limited to casein, corn flour, tapioca, sugar, honey, and butter. Sodium and potassium were given separately, as were supplemental vitamins. By these means, the magnesium intake was reduced to 1.1 mEq per day. As a preliminary study demonstrated that this diet alone was insufficient to cause a large negative magnesium balance, a cation exchange resin was added to the regimen.

Each subject withstood the depletion periods of 20 and 24 days without perceptible change. There were no alterations in blood pressure, pulse rate, electrocardiograms, basal metabolic rate, or maximal concentration of the urine. Each subject subsequently underwent a similar study, with the exception that a liberal intake of magnesium was added to the diet.

Magnesium depletion resulted in negative balances of 46 and 72 mEq, respectively. Initially, urine losses of magnesium were high, but by the twelfth day, the urine magnesium was reduced to 1 mEq/per 24 hours. No change in the serum magnesium level occurred.

During magnesium depletion there was no excess loss of nitrogen. This would suggest that magnesium did not come from the intracellular compartment, as the loss of this ion should result in breakdown of protein. As the total amount of magnesium in the body is about 2,000 mEq, of which one-half is in muscle, it is pertinent to ask if changes of the magnitude of 5 to 7 per cent would be detectable in a nitrogen balance. Conventionally, it is assumed that changes in nitrogen balance must exceed 10 per cent to be significant.

Magnesium depletion was associated with a gain of sodium and chloride. This was accompanied by a gain in weight relative to the control studies, and thus suggested expansion of extracellular fluid at this time.

Each study was terminated by the intravenous injection of magnesium. Twenty-five and 42 per cent of the injected magnesium was retained after the depletion studies, whereas none was retained after the control studies.

These well-conceived studies demonstrate the difficulties inherent in the study of magnesium deficiency. The body stores are large and renal conservation is efficient. The authors suggest that magnesium depletion called forth bone magnesium to replace the losses, but the evidence for this needs reinforcement. However, this relatively neglected aspect of human nutrition is now coming under active investigation, and answers to these questions will be forthcoming.

IDIOPATHIC HYPOPROTEINEMIA

Idiopathic hypoproteinemia is a diagnosis which can be made only after a thorough search has been conducted to exclude all known causes of low serum proteins. Among

many other conditions these latter include dietary insufficiency, gastrointestinal obstruction, pancreatic disease, chronic diarrhea, many types of liver disease, renal diseases accompanied by severe proteinuria, malignancy, hyperthyroidism, diabetes, and chronic febrile illnesses. In one of the early cases reported (D. A. Rytand, *Arch. Int. Med.* **69,** *251 (1942)*) a careful study revealed no apparent cause for the hypoproteinemia, but several interesting facts were established which have served as a basis for later investigation. Prothrombin and hemoglobin were normal, but serum albumin and globulin were reduced proportionately so that the ratio was normal.

By comparing the protein fractions, measured by electrophoresis, with recently published normal standards the total plasma protein was 73 per cent of normal, albumin was 70 per cent, fibrinogen was 78 per cent, and globulins, α, β and γ were 59, 82 and 92 per cent of normal, respectively. The changes in the formed elements of the blood including eosinophilia, reticulocytosis and increased thickness but decreased diameter of erythrocytes, along with low concentrations of uric acid and bilirubin, suggested that the bone marrow or reticuloendothelial system might be involved in this case of idiopathic hypoproteinemia.

In order to learn the nature of the metabolic abnormality operating in this unexplained serum protein defect, F. Albright, F. C. Bartter and A. P. Forbes (*Tr. Assn. Am. Phys.* **62,** *204 (1949)*) studied the fate of human serum albumin (52 g. daily) administered intravenously to a patient with idiopathic hypoproteinemia. The injected albumin was either (1) unchanged, (2) oxidized or (3) converted to protoplasm, as determined by the extent of reduction in phosphorus excretion. The fact that 65 per cent was oxidized and only 8 per cent was unchanged suggested that the defect was not decreased synthesis but rather increased destruction of plasma proteins

L. W. Kinsell *et al.* (*J. Clin. Invest.* **29,** *238 (1950)*) administered S^{35}-labeled methionine to a patient with idiopathic hypoproteinemia but failed to demonstrate reduced protein synthesis. Instead, they believed that the increased rate of disappearance of S^{35} from plasma protein and the increased urinary excretion of the isotope, as compared with that in normal control patients, indicated that "the basic defect is one of hypercatabolism".

The question of whether injection of large amounts of albumin (F. Albright, F. C. Bartter and A. P. Forbes) was unphysiological and the possibility that S^{35}-labeled methionine could be re-utilized leading to equivocal findings (Kinsell *et al.*), led M. Schwartz and B. Thomsen, (*Brit. Med. J.* **1,** *14 (1957)*) to study the serum protein turnover in a case of idiopathic hypoproteinemia using serum albumin labeled with I^{131}. That I^{131}-labeled human albumin remained tagged throughout the existence of albumin as an intact molecule, and that the labeled protein behaved *in vivo* like native protein, was tested by H. K. Sterling (*J. Clin. Invest.* **30** (**2**), *1228 (1951)*). Small amounts of I^{131}-labeled human albumin, mixed with thousandfold quantities of native human albumin, were injected into rabbits intravenously and plasma samples were collected on alternate days for two weeks. The approximation of the curves for disappearance of tagged albumin, and the immunological tests, indicated similar rate of disappearance of labeled and native albumin.

The patient studied by Schwartz and Thomsen had an eight year history of edema resulting from hypoproteinemia. Exhaustive physical examination and laboratory testing revealed no abnormalities other than a serum protein of 4 per cent, with albumin 2.6 per cent and globulin 1.4 per cent. There was a slight eosinophilia. Tentative treatment with a high protein diet, testosterone or corticotrophin at different times did not alter the serum protein value. This failure to alter the serum protein level by protein feeding was also seen in a similar patient

reported by C. L. Cope and H. K. Goadby, (*Lancet* **I,** *1038* (*1935*)). In their study the feeding of a low protein diet resulted in a negative nitrogen balance while a second period of feeding a high protein diet yielded a positive nitrogen balance, with the serum protein level being unaffected in either case.

Since the turnover rate of extrinsically, iodine-labeled proteins is more rapid than that of biologically-labeled proteins, and because *in vitro* labeling may induce subtle alterations which cannot be detected, Schwartz and Thomsen studied the albumin turnover in three normal persons as well as in the hyproproteinemic patient. In order to inhibit the uptake of I^{131} by the thyroid, a solution of potassium iodide was administered daily. Labeled albumin (30 mg. with a radioactivity of about 90 microcuries) was injected intravenously and heparinized blood samples were drawn and analyzed for radioactivity either every day or every other day for two weeks.

The radioactivity of the first sample, taken after 10 minutes, was considered as 100 per cent and was used for determining the plasma volume. Subsequent values were plotted on a logarithmic scale as percentages of this ten-minute value. The resulting graph was made up of two parts; a curved portion for the first five to six days represented a rapid dilution of the radioactivity, followed by a straight line representing the rate at which labeled albumin was replaced by unlabeled albumin. Using these curves the half-time of the administered albumin was computed as 22.7 days for the normal control patients and 7.2 days for the patient with idiopathic hypoproteinemia. The average albumin degradation was 173 mg. per kilogram of body weight in the control patients and 366 mg. in the subject. About 50 per cent of the injected dose of I^{131} was excreted by the control patients in the first 14 days, in which time the hypoproteinemic patient excreted 91.9 per cent. This indicated that the latter patient was degrading and synthesizing about twice the amount of albumin, per kilogram of body weight, as were the normal persons.

Schwartz and Thomsen pointed out that the achievement of balance at a low level of albumin "must be interpreted on the assumption that the albumin level *per se* influences the catabolism which must fall with decreasing albumin concentration, or presupposing a uniformly high albumin degradation regardless of the albumin concentration—that albumin synthesis in this condition increases with decreasing albumin concentration."

Although the mystery of idiopathic hypoproteinemia is not solved, it seems reasonable to propose that the basic defect is hypercatabolism rather than reduced synthesis.

PROTEIN MALNUTRITION IN BRAZIL

In the relatively few years during which protein malnutrition in infants has been studied systematically, it has become increasingly evident that the disease may take quite different forms under different environmental conditions. Thus, typical symptoms in one locality may be altered or even absent in another, depending on whether general undernutrition, specific vitamin deficiencies, infections, or parasitic infestations are complicating factors. For this reason, it has been important to treat each region as a separate entity and to study the disease in its relationship to the customs, dietary habits, climate, and prevalence of other diseases of the locale (*Nutrition Reviews* **13,** *67* (*1955*)). In this light the typical symptoms of kwashiorkor, as studied in Africa (*Ibid.* **10,** *261* (*1952*)) have been compared with those prevalent in similar populations of India (*Ibid.* **14,** *263* (*1956*)), Indonesia (*Ibid.* **12,** *72* (*1954*)), and Jamaica (*Ibid.* **13,** *140* (*1955*)).

Recently, J. Waterlow and A. Vergara

(*Bull. World Health Org.* **15** *165* (*1956*)) reported the results of a survey of protein malnutrition in Brazil undertaken in 1953 on behalf of FAO and WHO. The survey covered five cities and, where possible, the towns and villages near these cities. In Belo Horizonte, Recife and Rio de Janeiro, the condition, known as *distrofia pluricarencial* (multiple deficiency) was prevalent, whereas in Belem and Porto Alegre, it was rare. The survey was made with the help of local health authorities, school pediatricians and children's hospitals, and was concerned not only with protein malnutrition but with the general nutritional status of the population.

Although the condition of *distrofia pluricarencial* was generally similar to kwashiorkor, certain differences were apparent, and in many cases the symptoms were apparently intermediate between those of typical kwashiorkor and simple infant malnutrition (marasmus). The most prevalent symptoms were edema and weight loss, usually accompanied by one or more of the following: depigmentation of the hair (30 to 40 per cent of cases), dermatosis (50 to 60 per cent of cases), stomatitis (30 to 60 per cent of cases), glossitis (40 to 60 per cent of cases) and hepatomegaly (30 to 40 per cent of cases). The authors diagnosed the condition as kwashiorkor (or protein malnutrition) when weight loss or subnormal growth rate were accompanied by one or more of these symptoms.

In Brazil, as in Africa, the condition was most prevalent between the first and third years, although weaning appears to be much earlier in Brazil.

Since in Brazil, protein malnutrition is usually accompanied by general undernourishment, subnormal weight is a very common symptom, even though, in some cases, it is masked by edema. This latter condition was very prevalent and was usually accompanied by low serum albumin (as low as 4 g. per 100 ml.) and frequently by parasitic infestation, which, however, did not appear to be the main cause.

The hair changes in Brazil were not as prevalent or striking as in Africa. These changes usually consisted of dryness, thinning, and depigmentation to a reddish hue, especially in those of African descent.

Dermatosis, often resembling pellagra, also was dependent on racial descent, being most prevalent in the black children and least prevalent in the white children.

Hepatomegaly and fatty liver were common features of the disease in Brazil as in Africa, whereas cirrhosis and primary cancer of the liver were found to be rare in the *distrofia pluricarencial* in contrast to kwashiorkor in Africa.

Other accompanying symptoms (which may be due largely to vitamin deficiencies) are glossitis, cheilitis, and stomatitis. Rickets often appeared during recovery from the disease, probably because of the rapid growth induced by an improved diet.

Treatment followed the usual pattern change to a diet rich in protein (especially milk) transfusions in serious cases, penicillin (to prevent complications) and vitamins A, C and possibly the B complex. Other complementary measures are lipotropic substances, amino acids, human milk and diuretics. Even with the best treatment, however, the death rate may be as high as 30 per cent, and complete cure may take as long as 10 months. It is thus evident that a means of prevention should be sought as the simpler alternative.

The basic causes of the disease in Brazil seem to be too early weaning to a diet too low in protein. As in Africa, the staple diet is essentially vegetable (cassava, corn, or beans). The infant weaned at 3 to 6 months is particularly susceptible to prevalent diseases, and an attack of diarrhea may precipitate a state of malnutrition, especially since the mothers usually remove any eggs and milk from the diet under these conditions. Vitamin deficiencies and parasitic infestations also aggravate the disease at all stages.

Several measures are being undertaken

by the health authorities, with the hope of preventing the disease by raising the dietary protein. Although milk is rare and costly in the tropics, milk powder, especially defatted, is being supplied in increased quantities.

Fish and other sea foods should supply an increasingly greater portion of the diet (*see Nutrition Reviews* **12,** *72* (*1954*)). A change to vegetables richer in protein than the cassava or corn, such as soya, red bean, rice and others, is being attempted. It is especially necessary to educate the people into good nutritional habits and away from harmful customs such as the very early weaning and the dependence on a vegetable diet (*Ibid.* **13,** *129* (*1955*)). Improvement of the economic status and free supplemental food for pregnant and nursing mothers and infants should be of some help.

The authors point out that much research remains to be done on this disease, and they emphasize some of the areas such as the fundamental biochemistry, pathology and etiology, which are obscure at present. Many of the regions in which protein malnutrition is prevalent are too primitive for such research to be carried out. In Brazil, however, the facilities exist which should enable considerable advances to be made in the very near future.

BODY WATER

Quantitatively speaking, water is probably the most important nutrient and metabolite in the body. It is the major component of both the extracellular and intracellular compartments. Enzymatic reactions and electrolytic dissociation (with few minor exceptions) are possible only in the water medium. The measurement of the amount of extracellular and intracellular water in the living subject has been difficult, and rather little is known about the static and dynamic states of the different body fluids compared with our increasing knowledge concerning the composition of these fluids.

B. Friis-Hansen (*Acta paediat.* **46,** *Suppl. 110* (*1957*)) has reported observations conducted by him, both in this country and in Denmark during a period of several years, concerning changes in body water compartments during growth. The term "total body water" has been used by this author to denote the uncorrected volume of dilution of tracer doses of deuterium oxide. Details of the method used have been published (P. R. Schloerb *et al. J. Clin. Invest.* **29,** *1296* (*1950*)). The volume of the extracellular fluid compartment was determined using the thiosulfate method (Friis-Hansen, *Acta paediat.* **43,** *444* (*1954*)). The volume of the intracellular water was calculated by subtracting the extracellular water from the total body water.

The material collected in this monograph consists of measurements of total body water and/or extracellular water in 93 infants and children ranging from birth to 16 years of age. A total of 73 determinations of total body water and 51 measurements of extracellular water was carried out. In 31 cases in which both determinations were done, the intracellular water was calculated. For purposes of further analysis, the data on total body water were divided into five age groups: 0 to 11 days, 11 days to ½ year, ½ to 2 years, 2 to 7 years, and 7 to 16 years. No sex difference was found. The best correlation between total body water and the measures of body size was found to be a combination of weight and height giving a relative standard deviation of 7.9 per cent. However, correlation between total body water and weight alone, per cent of weight, or surface area was almost as high with relative standard deviations of 8.0, 8.1 and 8.2 per cent, respectively. There was poor correlation between total body water and height alone or age alone.

Friis-Hansen concluded that, for clinical purposes, the simplest estimate of total body

water was obtained by calculation in per cent of body weight within the different age groups. When calculated in this way, the total body water was found to be 77.6, 72.2, 59.5, 63.1 and 58.4 per cent of body weight, respectively, in the age groups listed above.

In order to illustrate the changes in total body water during the total life span, the corresponding values of total body water and body weight were plotted against each other on a double logarithmic scale representing subjects ranging from the first month of gestational age to adult size. Data from previous investigation were included with the present observations. This graph shows that a gradual drying-out took place which was most pronounced during the first year of postnatal life. In other words, the gain in total body weight during this period was more rapid than the gain in total body water.

The data for extracellular water were similarly analyzed. The correlations between extracellular water and weight plus height, per cent of weight, and surface area, were of the same order of magnitude with relative standard deviations of approximately 15.2 per cent. When the extracellular water volume was calculated as per cent of body weight the values for the age groups listed above were 41.6, 33.7, 26.2, 24.7, and 19.9 per cent. When expressed in this fashion the values for extracellular water volume show a gradual decrease throughout childhood. Extracellular water, expressed as liters per square meter of body surface, showed a remarkable constancy within the age groups: 6.7, 5.8, 5.9, 5.9, and 5.6 liters per square meter of body surface, respectively.

The calculated intracellular water volume was also expressed as per cent of body weight. The correlation with body weight was poorer than that for the determined values with a relative standard deviation of 20.8 per cent. The calculated values for the various age groups were 34.8, 37.9, 34.7, 39.9 per cent with only one determination for the oldest age group of 46.7 per cent.

Friis-Hansen has related his data to older studies in the literature, consisting largely of determinations of total body water by desiccation, and found good agreement. During prenatal life and continuing during the first year of postnatal life, there is a rapid decrease of total body water expressed as per cent of body weight. During this phase of growth there is a similar decrease of extracellular water with the intracellular water compartment remaining practically constant on a weight basis. Following a slight increase of all three compartments from 1 to 2 years of age there is a continuing decrease of extracellular water during the latter part of childhood, with only minor changes in total body water or in intracellular water. These changes have been interpreted as a relative increase in the proportion of cells in most tissues at the expense of extracellular fluid, a disproportionately higher development of such organs as muscles containing a high proportion of intracellular water, or a varying amount of fat tissue in the body. It should also be pointed out that the relative constancy during childhood of the volume of extracellular fluid when expressed on a surface area basis may partly explain the close correlation found between renal function and surface area in infants and children.

MAGNESIUM AND ELECTROLYTE EXCRETION

A lack of data regarding the effects of intravenous magnesium sulfate upon electrolyte excretion prompted B. I. Heller, J. F. Hammarsten, and F. L. Stutzman (*J. Clin. Invest.* **32,** *858 (1953)*) to study this problem. Normal subjects received 45 mEq of magnesium sulfate intravenously in 45 minutes and the renal hemodynamics, as well as electrolyte excretion, were compared with the control period.

There were only minor changes in renal plasma flow or glomerular filtration. Sodium

and chloride excretion were increased 48 per cent and 41 per cent, respectively, while potassium excretion decreased 44 per cent. Neither increased load nor osmotic diuresis was a factor in the natriuresis (excretion of sodium). The data were compatible with the hypothesis that magnesium may influence the exchange mechanism concerned with the tubular handling of sodium and potassium.

R. A. Womersley (*Clin. Sci.* **15,** *465 (1956)*) confirmed and extended these observations, examining the effects of infusions of magnesium sulfate, chloride and lactate upon the excretion of electrolytes. Inulin clearances were measured throughout the experiments, and after three control periods 20 to 40 mEq of the magnesium salt were given.

Following magnesium sulfate there was an increased excretion of sodium which was maximal in the first 15-minute period of the infusion. Subsequently, the rates of sodium excretion declined to normal. Magnesium chloride and lactate had no significant effects upon sodium excretion.

In all the studies with the three salts of magnesium, potassium excretion decreased and calcium output increased. These changes were maintained throughout and following the infusion. Inulin clearances were unchanged. The ratio of the clearances of magnesium and inulin was never higher than 0.22.

It appeared unlikely that the temporary natriuresis following magnesium sulfate was due to the magnesium ion, as the other salts did not cause this phenomenon. Osmotic diuresis could also be eliminated as a factor. It was suggested that the relatively poor absorption of sulfate forces the increased excretion of a cation, sodium.

The kaliuresis (excretion of potassium) seen, however, was probably due to the magnesium ion as the chloride and lactate were as effective as the sulfate. Since there was no change in the excretion of ammonia or titratable acid, it was necessary to postulate that magnesium decreased the tubular secretion of potassium without altering the secretion of hydrogen ion.

These observations suggested that a divalent ion, magnesium, was able to influence the tubular secretion of potassium, a monovalent ion. In order to examine this action further, F. O. K. Jabir, S. D. Roberts and Womersley (*Clin. Sci.* **16,** *119 (1957)*) determined the effects of an infusion of magnesium during the administration of a carbonic anhydrase inhibitor, acetazolamide. As it has been shown that inhibition of carbonic anhydrase results in an increased tubular secretion of potassium, it appeared possible that an infusion of magnesium would modify the action of acetazolamide.

These investigators demonstrated that ammonium chloride increased the excretion of magnesium and sodium but decreased the excretion of potassium. When acetazolamide was given immediately after the ammonium chloride, magnesium excretion dropped to control levels while sodium and potassium output increased. Although it is possible that the changes in magnesium excretion during ammonium chloride acidosis were due to an increased load as a result of a greater proportion of the serum magnesium becoming filterable, the increased acidosis due to acetazolamide led to a decreased magnesium excretion. Therefore, the changes in the handling of magnesium were probably due to tubular phenomena.

When acetazolamide was given during an infusion of magnesium, a normal potassium diuresis appeared. It would seem, then, that the effect of magnesium in causing a potassium retention must occur with some system in the tubular cell separate from the exchange mechanism. Confirmatory evidence was provided by showing that infusion of potassium had no consistent effect on magnesium excretion.

These studies have added to our knowl-

edge of the kidney's role in magnesium metabolism. The observations concerning the effects on potassium secretion may lead to the development of another tool to use in the investigation of potassium and tubular physiology.

DIETARY ENTEROCOCCI AND GROWTH OF CHICKS

It has been well-established that, under a variety of conditions, the growth of young chicks can be improved by including an antibiotic or a mixture of antibiotics in the diet (*Nutrition Reviews* **13,** *169* (*1955*)). Although no single mechanism will explain the different types of growth-promoting effects that have been reported, the one common feature underlying all of these observations appears to be the action of antibiotics in modifying the intestinal flora (H. D. Branion, G. W. Anderson and D. C. Hill (*Poultry Sci.* **32,** *335* (*1953*)).

The failure of antibiotics to stimulate the growth of chicks raised in quarters not previously used to house poultry suggested that one of the mechanisms of the growth-promoting action of antibiotics was the prevention of subclinical infections. This hypothesis appeared particularly plausible when it was observed that, unless antibiotics were included in the diet, the maximum weight attainable in an environment used regularly for experiments with chicks was less than that obtained with birds raised in new quarters (*Nutrition Reviews* **10,** *270* (*1952*)). Evidence indicating that antibiotics reduce the number of toxin-producing microorganisms (*Clostridium* species)in the intestinal tract of chicks (*Ibid.* **11,** *317* (*1953*)) provided further support for this hypothesis.

The feeding of certain *Escherichia coli* strains to chicks was found by G. W. Anderson, S. J. Slinger, W. F. Pepper, and M. V. Hauser (*Ibid.* **11,** *334* (*1953*)) to improve the rate of growth of chicks consuming a practical feed. This improvement in the growth rate was accompanied by an increase in intestinal coliforms and a decrease in enterococci. In following up these observations Anderson, Hauser, M. L. Wright, and J. R. Couch (*Canad. J. Microbiol.* **2,** *733* (*1956*)) have now determined the effect on the growth of chicks of feeding diets containing mixed enterococcus cultures. They have also studied the effect of the antibiotic chlortetracycline under these conditions.

The basal diet used in their investigation was a practical feed consisting of 10 per cent ground wheat, 42 per cent ground yellow corn, 30 per cent soybean oil meal, 2 per cent dehydrated green feed, 2.5 per cent meat meal, 3 per cent fish meal, 2.5 per cent dried buttermilk, 5 per cent tallow, 1 per cent ground limestone, and 2 per cent steamed bone meal. To this were added small quantities of iodized salt, vitamin A, vitamin D, methionine, choline chloride, manganese sulfate, riboflavin, calcium pantothenate, niacin, an arsonic acid, and vitamin B_{12}.

The experiment consisted of five duplicate groups of 22 male Columbian Rock chicks. A control group received only the basal diet. Two groups received the basal diet with a low, or with a high, level of a mixed culture of enterococci. The remaining two groups received crystalline chlortetracycline hydrochloride at a level of 40 g. per ton of diet (about 44 mg. per kg.), one with a high level of enterococci and one without this addition.

The mixed cultures of entercocci were prepared from organisms isolated from the cecal contents of chicks fed on a practical diet. Cultural, physiological, serological and chlortetracycline hydrochloride sensitivity tests carried out on the mixed culture used in the feeding trials indicated that three strains were present. These resembled previously described enterococci, but could not be

related directly to the specific strains that have been studied in detail. For the feeding trials 48-hour-old cultures, grown in broth, were centrifuged and washed in tap water. The cell suspensions were mixed into the diets daily at a 10 per cent level (v/w). The low level culture contained about 14×10^5 and the high level one contained about 28×10^9 cells per milliliter of suspension. These levels of the mixed cultures provided 0.51 per cent and 1.0 per cent, respectively, of dry matter in the diet.

The final average weight of the group receiving the high level of the mixed culture was significantly lower than that of the control group (270 g. compared to 352 g. at four weeks). The addition of chlortetracycline hydrochloride improved growth, both in the presence and in the absence of the high level microbial suspension, the average weights at four weeks being 375 g. and 422 g., respectively. The low level suspension of enterococci reduced growth slightly but not significantly.

The numbers of coliforms, lactobacilli, and enterococci per gram of duodenal, ileal, and cecal contents were estimated for each group. The counts for the group receiving the low level culture did not differ appreciably from the control values. However, there was an increase in the number of enterococci and a decrease in the number of coliforms, primarily in the ceca, of the birds receiving the high level culture. This trend was not evident in the group that received chlortetracycline with the high level culture.

The results demonstrate very clearly that feeding a high level of a mixed enterococci culture will retard the growth of chicks, and that this growth retardation can be prevented by including chlortetracycline hydrochloride in the diet. The full growth response to the antibiotic was not obtained when it was fed with the high level culture. Although a correlation between the rate of growth of the chicks and the numbers of enterococci in the intestinal tract was evident in these experiments, the results do not provide any information as to the immediate cause of the growth retardation caused by feeding enterococci (such as competition with the host for nutrients or the production of toxic products).

They do demonstrate, however, that the relationship between bacterial groups in the intestinal tract can be altered by dietary means. They also provide further evidence in support of the hypothesis that one of the mechanisms by which antibiotics stimulate growth is through controlling the growth of undesirable microorganisms in the intestinal tract.

THE NUTRITIVE EVALUATION OF FORAGES

Breeding investigations designed to improve the productivity of forage crops have been expanding rapidly in the United States during the past decade. The ultimate criterion of the value of a forage must include a measure of its nutritive value for animals. Accurate methods of evaluating feeds in the major forage-consuming animals (cattle) are therefore assuming increasing importance among animal nutritionists.

An excellent discussion of the problems inherent in nutrition studies and feed evaluation in ruminants has recently been published from the Pennsylvania Agricultural Experiment Station, University Park, (R. W. Swift, *Northeast Regional Publication, Bulletin 615*, (*January 1957*)).

The view is presented that more nutriment is required to maintain normal energy metabolism than for all other purposes combined. Considering the important crops intended for consumption as forages, there is a high degree of likelihood that if energy needs are satisfied, all other nutrient re-

quirements, with the possible exception of protein, will likewise be met. For this reason, energy content has served as the major basis for the compilation of rations for animals.

Recognizing the associative effects commonly encountered when both forages and concentrates, or supplements, are fed to ruminants, Swift believes that the relative nutritive value of various forage species must be secured from experiments in which forages alone are fed. (By associative effects is meant the influence of one feed constituent upon the digestibility, or net energy retention, of another feed constituent.)

Of primary importance, of course, is the decision as to what yardstick should be used to gain a relatively large amount of information on the nutritive quality of many forage species.

If one partitions the energy of feed into its compartments in animal metabolism, one would first consider the two major divisions; that which is removed during passage through the digestive systems (digestible energy), and that which is voided (fecal energy). The digestible energy can be further divided into urine energy, energy lost in the form of gas as a result of fermentation in the rumen (mainly methane), and the remaining fraction, which is referred to as metabolizable energy. The latter is accounted for either in heat production or in body gain. The body gain fraction is usually referred to as the net energy fraction of the feed, or its productive worth.

Net energy is clearly the ideal measure of the nutritive value of feed in terms of energy and would thus provide the most precise answer in forage evaluations. An appreciation of the work involved and the equipment required forces most investigators to a somewhat simpler, less expensive though less accurate, evaluation method. In considering the work involved in a net energy determination, it is clear that respiration equipment needs to be employed for determining fermentation gases expelled and for determining heat production indirectly from the respiratory gases, unless a large animal calorimeter is available.

In view of the above-mentioned limitations, the procedure most widely used in evaluating forages is a determination of the total digestible nutrients, a procedure devised at the Weende Experiment Station in Germany nearly one-hundred years ago and commonly referred to as the "TDN system."

To obtain total digestible nutrients (per hundred pounds of feed) the feed and feces are separated by empirical chemical analyses into component parts. It is necessary then to determine the digestion coefficients of each part (crude protein, crude fiber, nitrogen-free-extract, ether-extract, and ash) and then to multiply these values by the amounts of the consittuents in 100 pounds of feed. In the case of ether-extract, the product is multiplied by 2.25 to take into account its greater energy value. The sum of the products (per cent digestibility times the amount present of each constituent per 100 pounds of feed) is the TDN per 100 pounds of feed.

The short-comings of the "TDN system" have been adequately reviewed recently in two excellent reviews (L. A. Maynard, *J. Nutrition* **51,** *15 (1953)*; K. L. Blaxter, *J. Dairy Sci.* **39,** *1396 (1956)*).

One of the major deficiencies in the "TDN system" is that apparent digestibilities are measured without correction for fecal constituents of metabolic origin. Another error in the empirical determination of total digestible nutrients is encountered with feeds high in fat. Such fats may appear in feces as soaps which are insoluble in ether and hence are computed to be digestible. The analysis for crude fiber (boiling in dilute acid and subsequently in dilute alkali) bears no direct relation to the structural constituents of the material analyzed and no doubt the compounds analyzed by this procedure in feeds may be appreciably different from those analyzed in feces.

In spite of the limitations of the "TDN

system," its wide usage has established the evaluation of feeds on this basis on a broader scale than has any other method.

The obvious practical procedure proposed by Swift for the evaluation of forages is to determine the gross energy content of the feed and the feces and to express the differences between the two as digestible energy. Its determination is much simpler than a TDN determination, since it does not require that the feed or feces be separated empirically into its component parts. The only equipment required is a bomb calorimeter for gross energy determination. Why a simple gross energy determination, which is a highly accurate and reproducible laboratory procedure, has not attained wider usage, appears to be related to the fact that the bomb calorimeter was not in common use for many years subsequent to the development of procedures for the empirical chemical separation of feed components.

Metabolizable energy, defined as the gross energy of the feed minus the energy of the feces, urine, and methane, would obviously be a more refined and discriminating measure than digestible energy, which includes the waste energy of urine and methane. However, the wide use of metabolizable energy as a measure of nutritive value of forages cannot be foreseen because of the requirement of measuring accurately the fermentation gases.

As a result of the accumulation of considerable data at the Pennsylvania Station on net energy and metabolizable energy values of feed stuffs, it became possible to derive constants, or to develop methods of predicting energy losses through fermentation gases, and also to predict urine energy and heat loss. It was further possible to evaluate critically the relationship between TDN, digestible energy, and metabolizable energy.

The quantitative relationship between TDN and digestible energy was determined by assembling data on 48 experiments in which forage alone was fed and in which feed and fecal energy as well as TDN were determined.

The average digestible energy value for these forages was found to be 2018 calories per pound of TDN, and there was indication that the widely used value of 1814 calories per pound of TDN was too low when applied to forages.

A high correlation was found between TDN and digestible energy, the coefficient being 0.97. The TDN values could be computed from feed and fecal energy values (digestible energy values) with a possible error of only 2.8 per cent.

The relationship between digestible energy and metabolizable energy was also investigated, using data from forage-feeding trials in 35 experiments. The ratio of metabolizable energy to digestible energy was found to be .793 with a coefficient of variation of 2.6 per cent. Regression coefficients indicated that metabolizable energy may be computed from digestible energy with a possible error of only 2.3 per cent.

From these data Swift concludes that digestible energy can be determined with a minimum of time and experimental error and, thus, this procedure is the most practical means of determining the relative nutritive value of forages.

The results indicate that the metabolizable energy value of forages is about 79 per cent of the digestible energy value, indicating that about 21 per cent of the digestible energy is lost in fermentation gases and in urine. There is, of course, no comparable average value to express digestible energy in terms of gross energy of the forage. The Pennsylvania study indicates, however, that digestible energy varies between 35 to 75 per cent of the gross energy, depending upon forage species and stage of growth, and possibly other factors.

An important point is Swift's belief that forage evaluations should include both a digestible energy determination and a crude-protein digestibility determination. This is because protein appears to be the

the only nutrient likely to be deficient if the energy requirement is satisfied in a forage feed program.

It is hoped that the valid approach to forage evaluation suggested by Swift will stimulate other investigators to apply direct energy determinations in forage evaluation studies where the "TDN system" is currently widely applied.

A question worthy of consideration is the further application of the digestible energy measure in pasture evaluation studies where indirect methods must be applied to determine feed intake, and where it is desirable to derive digestibility data from samples of feces taken at random. The validity of such short-cut procedures has been reviewed (*Nutrition Reviews* **11,** *78* (*1955*)). Briefly, such procedures involve estimating pasture forage intake by administering known amounts of an indigestible tracer (chromium oxide) and determining its concentration in random fecal samples. Digestibility of the forage is calculated from the intake data and the observed quantitative relationship between chromogens (natural plant pigments) in the consumed forage and the voided feces. Twice-daily administration of the tracer was found to reduce the error of estimation of feed intake by this procedure (W. A. Hardison *et al.*, *J. Nutrition* **58,** *11* (1956)).

Of further interest is the recent caution that considerable energy loss occurs in drying ensiled forages and fecal matter as a preliminary step to energy determination in the bomb calorimeter (N. F. Colovas, H. A. Keener and H. A. Davis, *J. Dairy Sci.* **40,** *173* (*1957*)). The use of alcohol as a primer in determining the caloric value of fresh silages and moist feces eliminated the drying process and permitted accurate determination of gross energy on such materials.

DIETARY PROTEIN AND EXPERIMENTAL TUBERCULOSIS

As a part of a long-term study of the effects of nutritional imbalance on the response of the experimental animal to tuberculous infection, H. L. Ratcliffe and J. V. Merrick (*Am. J. Path.* **33,** *107* (*1957*)) have published convincing evidence that moderate protein deficiency materially alters the course of this disease in the hamster. Infection was induced by means of an inhalation device which insured a constant dose of organisms delivered to the lungs (W. F. Wells, H. L. Ratcliffe, and C. Crumb, *Am. J. Hyg.* **47,** *11* (*1948*)).

The animals were fed the experimental diets starting at about eight weeks of age and were maintained on the diets for six weeks before they were infected with small doses of H37Rv tubercle bacilli. Three diet groups consisting of about 50 hamsters each were studied with protein from vegetables and casein making up 6, 17, and 30 per cent of each isocaloric ration. Evidence indicating that the animals fed the low protein ration were not severely deficient was furnished by the fact that the animals in the 6 per cent protein group maintained their weight at 85 to 90 per cent of that of the animals fed the 30 per cent protein ration.

Groups of hamsters were killed for study at frequent intervals from 75 to 200 days after infection. Gross study of the distribution, size, and number of the tubercles in each animal, and microscopic evaluation of the reaction of the host to the infection as well as the status of the infective agents in the lesions, were carried out. There was essentially no difference in the course of the infection, grossly or microscopically, among the three diet groups during the first 130 days of the infection.

However, after this point, many of the animals fed the low protein ration, and to a lesser extent those fed the 17 per cent protein diet, showed a progressive and

widespread tuberculous infection spreading from the lung to many other organs and associated with precipitous losses in body weight. During this same period the majority of the hamsters receiving the 30 per cent protein ration showed regression of their disease, with smaller lesions than at 75 days. Late in the experiment, at about 200 days, some of the hamsters fed the high protein ration showed a progressive renal glomerulosclerosis. These animals frequently developed reactivation of their tuberculosis apparently in association with this kidney lesion.

H. L. Ratcliffe and J. V. Merrick discuss their results in relation to mechanisms of resistance to tuberculosis. They interpret an early period of the infection, during which the differing diets had little effect on the course of the infection, as the period of predominantly intracellular growth of the tubercle bacilli. The later stage of the disease, when the low protein diets seemed to cause a definite decrease in resistance to the infection, was the stage when the organisms were growing extracellularly to a greater extent.

Although there have been numerous observations in man which have suggested a correlation between nutritional state and resistance to tuberculosis, it has usually been difficult to separate the effects of the various dietary components. In fact in many instances poor hygiene, crowded living conditions, and other factors could not be ruled out. The study by Ratcliffe and Merrick appears to demonstrate a favorable experimental approach which should help to establish the dietary factors influencing tuberculous infection.

ASCORBIC ACID REQUIREMENT WITH PERIODIC ADMINISTRATION

Ascorbic acid, in common with the other water-soluble vitamins, appears to be poorly stored (or rapidly destroyed), and consequently must be supplied almost daily for efficient utilization. This method of administration is not always practicable, and in many cases it would be desirable to administer the vitamin at less frequent intervals. However, until recently, quantitative data on the relative effectiveness of ascorbic acid when given daily or less frequently have not been available. S. N. Matsko, V. I. Gorbunova, and A. T. Zhmeido (*Biul. Eksp. Biol. Med. (Russia)* **41,** *22 (1956)*) recently carried out three experiments designed to solve this problem.

Experiment I consisted of five groups of male guinea pigs, with 17 to 20 animals in each group at the start of the experiment. The diet prior to the experiment consisted of oatmeal and hay, supplemented with carrots and cabbage ad libitum. Experiment II consisted of five groups of 16 to 17 guinea pigs each, on the same basic diet with supplements of 0.25 mg. of vitamin A and 15 I.U. of vitamin D (as irradiated ergosterol) daily. In experiment III, with 31 to 33 guinea pigs in each of three groups, the same supplements given in experiment II were used with a basic diet consisting of oats, hay, and bran.

In each experiment, graded doses of ascorbic acid in water were given to each group of animals and the weight gain response was measured for 84 days. At this time, hemoglobin concentration was also measured in all animals. A significant relationship was shown between weight gain and daily dose of ascorbic acid for groups 1 to 4 (0.5, 1, 4, and 16 mg. per day) in experiments I and II, and for experiment III (1, 10, and 20 mg. per day of ascorbic acid for groups 1, 2, and 3). Similarly, the differences in hemoglobin were significant for the groups in experiments I and II. The significance of experiment III is in doubt, since many of the animals died of pneumonia during the experiment. However,

the number of deaths (38 per cent of group 1, 21 per cent of group 2, and 3 per cent of group 3) appeared to be inversely related to the amount of ascorbic acid supplement.

In experiments I and II, groups 5 were given doses of ascorbic acid equivalent to 16 mg. per day twice weekly, while groups 6 were given the same total amount once a week. In the groups 6, growth rates were significantly below those of groups 4 (16 mg. of ascorbic acid daily) and were about equal to those of groups 3 (4 mg. of ascorbic acid daily). The implication is that ascorbic acid, when given at weekly intervals, loses about three-fourths of its potency. Similarly, the doses given at bi-weekly intervals were about one half as effective as the same total given daily.

In vitamins which are readily stored, no such decrease in potency is observed. Thus, the authors found that vitamin A was as effective in weekly as in daily doses. In the case of ascorbic acid, even large increases in the dosages given at the less frequent intervals did not make them more effective. Under the conditions of these experiments, 20 mg. seemed to be about the maximum useful single dose for guinea pigs. A 30 mg. dose even resulted in a slightly lower weight gain.

These experiments have demonstrated three points: 1) Less frequent doses of ascorbic acid are not as effective as the same total amounts given daily; 2) By increasing the doses by specified amounts, they can be given less frequently; 3) Single doses cannot exceed a definite amount if they are to be effective.

POTASSIUM AND NITROGEN BALANCE

The relationship between potassium utilization and nitrogen balance has been studied by L. E. Frazier, R. H. Hughes and P. R. Cannon (*Am. J. Clin. Nutrition* **4**, *655 (1956)*) in order to determine whether potassium repletion can be attained during periods of negative nitrogen balance. Previous work had shown that potassium was required for protein repletion (*Nutrition Reviews* **12**, *107 (1954)*).

The authors conducted four balance experiments with normal and protein depleted rats. During the first period of each experiment the groups of five to ten rats were fed on a protein-free purified diet from which potassium had been omitted. In subsequent periods the nitrogen intake, the potassium intake, or both, were varied. Urine and feces were collected daily, or on alternate days, throughout the experimental periods which were 12 to 24 days in duration. The excreta were analyzed for potassium by a flame photometric method, and for nitrogen by a micro-Kjeldahl procedure.

The first two experiments, one with protein-depleted rats and one with normal rats, consisted of three periods of four or five days each. During the first period, when the animals received no nitrogen or potassium, they lost weight and were in negative potassium balance. During the second period when they were given daily subcutaneous injections of 250 microequivalents of potassium they continued to lose weight. The protein depleted animals showed a positive potassium balance on the first day, but by the fourth day this had disappeared and the potassium balance had again become negative. In the normal rats, not even a transient positive potassium balance was evident. When, in the third period, a nutritionally adequate amino acid mixture was included in the diet and the potassium injections were continued, both groups of animals gained weight rapidly and went quickly into positive potassium balance.

The third experiment consisted of a forced-feeding experiment with normal rats in which food intake was rigidly controlled and both nitrogen and potassium balance were de-

termined. As before, when the diet lacked the two elements, negative balances were observed for both of them. When the amino acid mixture was included in the diet during the second period, the nitrogen balance became positive, even though the animals received no potassium. The potassium balance remained negative but the potassium loss was reduced. Again, when both nitrogen and potassium were given, positive balances for both were obtained. However, during a subsequent period when tryp[illegible]han was omitted from the amino acid mixture, the nitrogen balance, as would be expected, became negative and the animals, in spite of the injection of 250 microequivalents of potassium daily, went into negative potassium balance after a lag of two days.

In the final experiment, two groups of protein depleted rats were given 1.0 or 2.5 mEq of potassium with, and without, the complete amino acid mixture. With these higher levels of potassium, positive potassium balance was obtained for four days when the animals were in negative nitrogen balance. However, by the sixth day, the potassium balance approached the zero line. The inclusion of the complete amino acid mixture at this point resulted in a prompt and dramatic retention of potassium.

These experiments, as the authors state, "indicate that the metabolic state of the tissues largely determines their ability to retain and utilize potassium ion." They also point out that the administration of potassium alone is unlikely to lead to any significant retention of potassium if tissue loss is proceeding actively. The necessity is also emphasized of achieving positive nitrogen balance in order to insure potassium retention in patients suffering from severe potassium loss. The logical design of these experiments, and the clear-cut results obtained, provide convincing support for the authors' conclusions.

UTILIZATION OF UREA BY THE RAT

At least three groups of investigators have reported that the growth of rats fed on diets containing only the indispensable amino acids is stimulated upon supplementation of the diet with a nonprotein nitrogen source, such as urea or ammonium citrate (*Nutrition Reviews* **9,** *109 (1951)*; D. V. Frost and H. R. Sandy, *J. Biol. Chem.* **189,** *249 (1951)*). These compounds presumably provide nitrogen for the synthesis of the dispensable amino acids which become limiting for growth when the diet does not contain them. Although there can be no doubt about the growth response in these cases, earlier other workers (M. Kriss and L. F. Marcy, *J. Nutrition* **19,** *151 (1940)*; K. Bloch, *J. Biol. Chem.* **165,** *469 (1946)*), who administered urea orally to rats receiving an adequate diet, were unable to demonstrate any growth response and found that most of the urea was excreted apparently unchanged. In order to obtain some direct evidence regarding the fate of urea under both sets of conditions, W. C. Rose and E. E. Dekker (*J. Biol. Chem.* **223,** *107 (1956)*) have reinvestigated this problem using N^{15}-labeled urea.

The experimental animals used consisted of two groups of three male rats each, all of which were from the same litter. One group received an adequate diet containing 18 per cent of casein, the other received a similar diet in which the casein was replaced by a mixture of the indispensable amino acids in quantities sufficient to meet the normal requirements. The group receiving the amino acid mixture was fed *ad libitum* throughout the experimental period which consisted of a 12-day preliminary period followed by a seven-day period during which N^{15}-labeled urea was included in the diet. The rats receiving casein were fed *ad libitum* for 16 days, then, when they were given the diet con-

taining the labeled urea, they were pair-fed so that they consumed the same quantity of food as the first group over the same length of time. Urine and feces were collected after the addition of urea to the diets and the collections from each group were pooled. The three carcasses of each group were combined. Urea was isolated from the urine samples. Tyrosine, cystine, proline, histidine and glutamic and aspartic acids were isolated from the carcass proteins. The isolated compounds, carcass proteins, urine, and feces were analyzed for N^{15}.

The results showed that the carcass proteins of the rats receiving only the essential amino acids contained almost 22 per cent of the isotopic nitrogen from the urea, while those of the group receiving casein contained only 3 per cent. The urine and feces of the former group contained 40 and 14 per cent while those of the latter group contained 81 and 2 per cent, respectively, of the labeled urea nitrogen. The authors conclude that "the nitrogen of urea can be utilized when the subjects are in need of this element for synthetic purposes". This is borne out by the results of the analyses of the individual dispensable amino acids, which indicated that the concentration of N^{15} was 14 to 16 times as high in the amino acids isolated from rats fed on the diet containing only indispensable amino acids as it was in those from rats that were fed on the diet containing casein.

Two additional points of interest emerge. One is that tyrosine, which is presumably formed from phenylalanine, was quite highly labeled. The other point is that histidine, an indispensable amino acid, did in fact contain (although relatively much less) one-fifth to one-tenth as much isotropic nitrogen as the dispensable amino acids. The labeling of these two compounds can, of course, as the authors point out, be accounted for by continuous deamination and amination within the animal body as observed by R. Schonheimer, D. Rittenberg and A. S. Keston (*J. Biol. Chem.* **127,** *385* (*1939*)). The higher labeling of the histidine and tyrosine in the proteins of the rats on the less adequate diet would suggest that this process is intensified when all the dispensable amino acids must be synthesized. Further support for such a mechanism is obtained from the results of R. Steele (*J. Biol. Chem.* **198,** *237* (*1952*)) who observed that when C^{14}-labeled sucrose was fed to rats, labeled carbon was present in most of the dispensable amino acids but could not be detected in the indispensable amino acids or in tyrosine.

The paper concludes with the observation that the experiments do not explain how the urea nitrogen becomes available for the use of the animal, but it is assumed that, as has been shown by R. Z. Dintzis and A. B. Hastings (*Proc. Nat. Acad. Sci.* **127,** *385* (*1953*)), urea is decomposed by the action of gastrointestinal organisms thus making ammonium nitrogen available for absorption.

Although in a previous study (W. C. Rose and L. C. Smith, *J. Biol. Chem.* **187,** *687* (*1950*)) no evidence was obtained from growth experiments that the synthesis of dispensable amino acids was depressed by feeding sulfa drugs, the question of the availability to nonruminants of amino acids synthesized by intestinal microorganisms is still open. The use of isotopic carbon compounds as dietary precursors of amino acids, as used by Steele, would be preferable to the use of nitrogen-labeled compounds for such studies. This would eliminate the complication of N-exchange reactions which occur in the tissues. If, after feeding the labeled compounds under a variety of conditions which are known to affect intestinal synthesis of vitamins (e.g., with various types of dietary carbohydrates and antibacterial agents in the diets), the label was not incorporated into the carbon skeleton of amino acids which cannot be synthesized by the tissues, it would be evident that intestinal synthesis of amino acids is not of significance in the nutrition of the nonruminant.

MINIMAL RESECTION OF THE SMALL INTESTINE AND ABSORPTION OF VITAMIN A

The effects of massive resection of the small intestine in decreasing the absorption of foodstuffs have been reviewed earlier (*Nutrition Reviews* **10,** *202 (1952)*). Recently M. R. Loran, T. L. Althausen, and E. Irvine (*Gastroenterology* **31,** *717 (1956)*) have studied the effects of removing only minimal amounts (approximately 10 per cent) of the small intestine on the absorption of Vitamin A by the rat. In this work the absorption of Vitamin A was being used as an index of the absorption of fat by these rats.

To carry out the study, male rats weighing between 300 and 350 g. were subjected to a resection of 10 to 15 cm. of the small intestine. This is approximately 10 per cent of the entire length of the small intestine. The continuity of the intestine was reestablished by joining the cut ends by an end to end anastomosis. To obtain information on the effect of the site of resection in one group of rats, the segment was removed from the upper end of the jejunum and, in another group, it was removed from the lower portion of the ileum. The vitamin A absorption test was carried out by giving the animals 1000 I.U. of vitamin A concentrate in olive oil per kilogram by stomach tube. The vitamin A used was in the ester form. The serum level of vitamin A was determined using a microspectrophotometric method both before, and four hours after, the oil was administered. The level of vitamin A in the blood four hours following the administration of the test dose of vitamin A was used as an index of the rate of absorption of vitamin A. The greater the blood level, the greater was the rate of absorption. The animals were tested two, eight, and 14 weeks after the resection was made in the upper small intestine, and three or four weeks after resection of the lower ileum.

The growth curves of the animals which had a 10 per cent resection of the upper jejunum were essentially the same as those of the unoperated control animals, indicating that these animals were absorbing sufficient amounts of nutrients for adequate growth. No evidence of a vitamin A deficiency was noted. These investigators found that to produce even a transitory postoperative growth inhibition, at least 50 per cent of the small intestine had to be removed. Even after removal of 65 per cent of the small intestine there was a normal growth in some of the animals. It should be noted that 300 g. rats do not grow at a rapid rate, and may be relatively insensitive to factors which might influence body weight during a state of more rapid growth. The control animals showed an average increase of the vitamin A level in the serum of about 28 micrograms per cent four hours after receiving the test dose. Two weeks after surgery, the animals that had resections of the upper portions of the small intestine showed increases of only approximately 15 micrograms per cent in the serum vitamin A levels after receiving the test dose. This increment of the serum vitamin A seemed to decrease progressively eight and 14 weeks after the operation (10.8 and 7.3 micrograms per cent, respectively), though the decreases were not statistically significant. The minimal resection of the upper small intestine of rats, removing only about 10 per cent, caused about a 50 per cent decrease in the serum vitamin A after a standard test load. This degree of inhibition in the rate of vitamin A absorption persisted for at least three and one-half months following the operation. Similar results were found when the resection was performed in the lower ileum, though the values are given only for a three to four week postoperative period. J. A. Benson, Jr., G. N. Chandler, F. E. Van Steenhuysen and J. O. Gagnon (*Ibid.* **30,** *53 (1956)*) have presented data obtained by measuring the absorption of olive oil tagged with I^{131}, that shows that resection at any level of the small intestine leads to a reduction in the absorption.

Following resection of either the upper or

lower small intestine Loran, Althausen and Irvine carried out studies using two emulsified vitamin A preparations. One had particles whose size was greater than 8 microns, and the other had particles of less than 8 microns in size. If the particle size of the fat emulsions was less than 8 microns, there was increased absorption of vitamin A in both the normal control animals and the animals with resections of the small intestine. The increase in the vitamin A levels of the serum in the control animals was 45 per cent above that found in normal animals four hours after receiving a non-emulsified vitamin A fat preparation. The resected animals showed increments of 139 per cent over that found in animals which did not receive the emulsified fat. Thus by emulsifying the vitamin A preparation, resected animals were able to absorb the vitamin A at a rate approximately equal to that of the control animals receiving the emulsified fat, and better than the non-operated animals receiving the vitamin A preparation in a non-emulsified oil preparation. If the emulsion was made so that the particles had a diameter greater than 8 microns, then there was an increased absorption in the normal animal but not in the resected rats. These latter animals showed absorption rates equivalent to those which were found when they received vitamin A in the form of non-emulsified oil.

On autopsy, the animals who had an intestinal resection were found to have greatly enlarged intestines. The increased diameter was not due to obstruction, since no stricture was found and the enlargement occurred both below and above the point of anastomosis. On microscopic examination the intestines of these animals showed a true hypertrophy. No differences in the morphology of the mucosal cells were noted on routine sections.

The increased absorption of vitamin A in the resected animals which received the test dose in finely emulsified form, indicates that mucosal cells in resected animals can handle the fat properly if it is presented in a form which can enter the mucosal cell. This also might indicate that there is impaired emulsification of fats in the resected rats, which could implicate either one or all of the following factors: secretion of bile, secretion of mucus, or secretion of pancreatic juice. On the other hand, there was anatomical alteration of the intestine as indicated by hypertrophy following the resection, and this might be a factor influencing the intestinal absorption of vitamin A.

This work indicates that even minimal resection of the small intestine will influence the absorption of fats, as indicated by the absorption of vitamin A. But the exact cause of this defect, whether it is anatomical or dependent on secondary influences (e.g. poor emulsification) is not established at present. However, the defects are subtle enough so that growth of the animal is not influenced.

BONE EROSION DURING LACTATION

It is well established that the reproductive cycle in mammals causes a considerable drain on the calcium and phosphorus reserves of the maternal organism, which usually remains in negative calcium balance even when receiving a high-quality diet. The reserves are then replenished before the period of maximum drain in the next cycle (J. Duckworth and R. Hill, *Nutr. Abstr. & Rev.* **23,** *1 (1953)*; R. Hill, *Agricultural Progress* **29,** *98 (1954)*).

A further study on the effects of diets low in calcium, has now been reported by D. Benzie *et al.* (*J. Agr. Sci.* **46,** *425 (1955)*; **48,** *175 (1956)*). This has an immediate application to the feeding of sheep, but the comparative evaluation of various technics (blood analysis, body ash, bone composition

and bone desity by x-ray photography in the live animal and dissected carcass) is of wider interest.

In the first experiment 36 mature ewes were transferred, when eight weeks pregnant, from good practical rations to individual indoor pens. Twelve animals (Group A) then received a daily allowance of 400 g. of chopped hay, 400 g. of a concentrate mixture (ground corn 85%, peanut meal 14% and sodium chloride 1%), and 2.5 ml. of cod liver oil. The concentrate allowance was later increased to 800 g. (during late pregnancy) and then to 1,200 g. (during lactation). This ration provided 2.0 g., 3.3 g., and 4.6 g. of phosphorus in its three stages, quantities which are close in each case to the estimate of requirements for sheep in mid and late pregnancy and lactation respectively. However, the provision of calcium was only 1.7 g., 1.8 g., and 1.9 g. per day respectively, compared with estimated requirements of 3.4 g., 4.5 g., and 4.6 g. per day at these times. Groups B and C received the same rations supplemented with 2.9 g. and 5.8 g. of calcium in the form of calcium carbonate, so as to allow an intake respectively close to, and considerably above, the estimated requirement.

There were only slight differences in the weight and initial growth rates of the lambs born to the three groups. After 100 days of lactation the ewes were killed, their skeletons were dissected, and all but the smallest bones were individually ashed. The mean ash weight for the total skeleton in group A was 890 g., a figure significantly below the figures of 1,050 g. and 1,100 g. obtained for groups B and C. Among individual bones the loss of mineral by group A was most strikingly seen in the vertebrae series, many of which had lost 25% of their mineral content, as compared with the corresponding animals that had received the highest level of calcium (Group C). In contrast, there was much less difference in the bones of the limbs; the metacarpal was apparently quite unchanged in its mineral content. The ribs and pelvic bones were affected to an intermediate extent.

These relations were to be expected in view of earlier findings with the rat, indicating that bones rich in cancellous tissue were subject to heavier drafts than those composed predominantly of compact tissue (G. M. Ellinger, Duckworth, A. C. Dalgarno, and M. H. Quenouille, *Brit. J. Nutrition* **6**, *235 (1952)*). This view was confirmed by the ash weights of the shaft and ends, respectively, of the humerus, femur and tibia that had been sectioned in a standard manner. In each case the ends of the bones, containing a high proportion of cancellous tissue, had a significantly higher ash content in group C than group A. There was no such difference in the shafts, which were mainly compact tissue.

The percentage of ash in the bones changed much less than the total weight. However, variation within a group was also less, so that, in general, significant differences in ash percentage of bones corresponded with significant differences in total ash content.

The concentration of calcium in the serum of individual sheep was measured at successive stages of the experiment. In group A, the mean level decreased, from an initial level of 11 mg. per 100 ml. to 8 mg. at parturition. It rose to 9 mg. in early lactation, and declined again to 7.5 mg. per 100 ml. in late lactation. The other groups showed changes of the same type, but of a smaller magnitude, so that differences between all three treatments are significant. However, any attempt to diagnose the adequacy of an animal's intake of calcium without reference to its exact stage in the reproductive cycle could be misleading. No significant differences were found in the level of inorganic phosphorus in the serum.

Radiographs were made of dissected flesh-free bones from the slaughtered ewes. These again showed a deficiency of cancellous tissue in some regions of the bones from the low-calcium group (A), and a more open

reticulous structure. The shafts of certain long bones from this group also showed some characteristic striations that were not seen in the other groups. As was to be expected from the analysis of ash content, the treatment differences were most obvious in vertebrae, pelvis, and ribs, and least obvious in the metacarpal and metatarsal (which are, unfortunately, those most convient for x-ray in the live animal).

In a second experiment of similar design, only the two lower levels of dietary calcium were used (*i.e.* corresponding to treatments A and B in the first experiment); also, some animals were killed as initial controls, others were killed at mid-lactation, and the remainder were killed two months after the end of lactation. Those killed as controls had 1,260 g. of ash in their skeleton. The corresponding figures at mid-lactation were 81% and 93% of this figure for treatments A and B, respectively. The corresponding figures after two dry months were 87% and 101%. Thus the erosion on the skeleton during pregnancy and lactation had not been made good, on the lowest level of calcium intake, during two months under maintenance conditions, in contrast to the complete restoration of reserves on treatment B (*i.e.* higher calcium intake) during the same period.

This conclusion was confirmed by the ash weights and radiographs of individual bones. In this trial radiographs were also taken of several regions of the live animal, but the quality of the pictures obtained was not adequate for confident discrimination between the different treatments. This was the case even when examination was concentrated on those bones which were more extensively resorbed than indicated by the 20% loss of total skeletal ash in treatment A, and when powerful x-ray equipment was used allowing an exposure period of 0.1–0.5 seconds.

At its present stage, this work has provided a considerable extension of knowledge about the withdrawal of mineral from the bone during reproduction on a low-calcium diet. However, the results have been disappointing, though salutary, as to the information that can be obtained by technics such as blood analysis and radiography which might be used in field studies, as compared with the analysis of dissected bones. There is of course particular difficulty in the radiography of sheep since the bones of the legs, which can be photographed without breathing movements and the passage of the fleece, are those least sensitive to erosion.

Lastly, it is to be emphasised that these studies should not be considered as evidence of the inherent danger of bone erosion during lactation. Rather, they confirm the physiological role of the skeleton as a mineral reserve, demonstrating that erosion can be accompanied by normal, healthy suckling of the young, and that, on a diet of normal (not excessive) calcium content, the reserves can be replenished in a short period. The test for the adequacy of a diet must, in fact, be whether or not the reserves can be completely replenished before the normal time for the onset of the next period of demand on the skeleton.

VITAMIN D AND CORTISONE—SYNERGISTS OR ANTAGONISTS?

There is conflicting evidence regarding the requirement of Vitamin D for local calcification of bone matrix independent of its function in promoting the absorption of calcium. Nevertheless, the end result of vitamin D deficiency is a failure of calcification of the bone matrix which continues to grow and during which there is a continuation of normal bone resorption.

The action of cortisone on bone metablism was foreshadowed by early observations of bone changes accompanying Cushing's

disease. H. A. Sissons (*J. Bone Joint Surg.* **38 B,** *418* (*1956*)) interpreted his observations of these changes to mean that hyperactivity of the adrenal cortex produced an inhibition of osteoblastic bone formation rather than an exaggeration of osteoclastic bone destruction. In fact H. N. Green (*Brit. Med. J.* **II,** *1165* (*1950*)) believes that this and many other actions of cortisone may be explained on the basis of its inhibition of cell mitosis.

Another aspect of the effect of cortisone on vitamin D and calcium metabolism has been reported by H. G. Morgan, R. G. Mitchell, J. M. Stowers and J. Thomsen (*Lancet* **II,** *270, 925* (*1956*)). These workers studying calcium metabolism in infants with idiopathic hypercalcemia observed marked reduction in serum calcium, doubled fecal excretion of calcium, and increased urinary calcium output following the administration of 25 mg. of cortisone daily.

H. A. Sissons and G. J. Hadfield (*J. Anatomy* **89,** *69* (*1955*)) observed that cortisone (10 or 20 mg. per kg. of body weight) injected in rabbits prevented longitudinal growth of bone and caused narrowing of the epiphysial cartilage plate and reduction in the number of metaphysial bone trabeculae. Osteoblasts were reduced in number but osteoclasts were numerous. The findings in rats were similar but not as marked. In one large series of rats studied in London there was progressive reduction in the number of metaphysial bone trabeculae, but in a smaller series studied in Chicago there was a contrasting increase in density of metaphysial bone near the epiphysial plate. This latter phenomenon also seen in rats by R. H. Follis (*Proc. Soc. Exp. Biol. Med.* **76,** *722* (*1951*)) was explained as the result of retardation of normal osteolytic sequences in metaphysial bone with consequent increased bone density.

H. Hanssler (*Klin. Wchnschr.* **34,** *646* (*1956*)) studied the effect of cortisone in conjunction with rachitogenic diets fed to 4-week-old rats, noting changes in thymus and spleen, serum values for calcium and phosphorus, and the progress of calcification of the tibia. The effect of adrenalectomy on the vitamin D_3 prevention of rickets was also observed. In some of the experiments the rachitogenic, Steenbock diet number 2965 was used. As usual the injection of cortisone caused involution of thymus and spleen in all daily doses ranging from 0.5 to 2.5 mg. per rat. Serum phosphorus was reduced by cortisone but was restored by the combination of cortisone with a prophylactic dose of vitamin D_3 whereas serum calcium remained unchanged. Cortisone in ordinary doses produced a narrowing of the cartilaginous zone, disappearance of osteoid and decalcification of newly formed bone spicules. In contrast to the rachitic control animals, the growth fissure was narrowed and broad densely calcified bands appeared on the metaphysis. The so-called tetany-producing diet of György, with a calcium to phosphorus ratio of 1:4 was used in later experiments. The serum calcium value was reduced below 6 mg. per cent and rickets was produced which was characterized by broadening of the cartilage zone, moderate osteoid formation, and in the lateral marginal regions some provisonal cartilage calcification. Cortisone caused constriction of the cartilage zone, thick calcification of the metaphysis, and normal provisional calcification of cartilage cells near the metaphysis. In the adrenalectomized rachitic rats prophylactic dosage with vitamin D_3, although able to restore the blood calcium and phosphorus levels, was unable to prevent the development of rickets.

Hanssler believes that cortisone plays an essential role in the normal growth of bone but that this is especially so for the process of ossification near the epiphysis. The study of the rates of bone accretion and resorption using radioactive phosphorus, reviewed recently (*Nutrition Reviews* **15,** *70* (*1957*)) emphasizes the concept that the final metabolic effects on bone must be the result of these two rates. The action of

cortisone would be expected to be inhibition of both osteogenesis and osteoclasis resulting from the inhibition of mitosis of osteoblasts and osteoclasts. This would result in cessation of growth, but neither shortening of bone nor increased deposition of calcium salts would be expected from this cause. Future experiments are needed which will define more critically the roles of vitamin D, cortisone and the serum levels of calcium and phosphorus in the intermediary metabolism of bone tissue.

VITAMIN D AND CITRATE OXIDATION

It has been recognized for many years that vitamin D exerts profound effects on the metabolism of calcium and phosphorus (*Nutrition Reviews* **14,** *253* (*1956*)). Although a relationship between calcium and citric acid also had been shown by early investigations, it was not until 1943 that a direct relationship was suggested between vitamin D and the metabolism of citric acid (R. Nicolaysen and R. Nordø, *Acta physiol. Scandinav.* **5,** *212* (*1943*)). Subsequently, it was shown that the administration of physiological doses of vitamin D to normal or rachitic rats led to an increased urinary excretion of citric acid (S. A. Bellin and H. Steenbock, *J. Biol. Chem.* **194,** *311* (*1952*)) and to increased concentrations of citric acid in the blood and bones (A. Carlsson and G. Hollunger, *Acta physiol. Scandinav.* **31,** *317* (*1954*)).

An explanation for this effect of vitamin D on citrate levels has been sought at the enzyme level of metabolism by H. F. deLuca, F. C. Gran and Steenbock (*J. Biol. Chem.* **224,** *201* (*1957*)). Young male rats were fed semi-purified diets containing glucose, heated egg albumin, cottonseed oil, vitamins (except vitamin D) and salts which were free of calcium and phosphorus. The desired levels of these two elements were then added to make the diets rachitogenic or non-rachitogenic. With each diet used, half the rats received orally 75 I. U. of vitamin D (calciferol) per rat every third day. After 12 to 15 days, the rats were killed, homogenates were prepared from the kidneys, and the oxidation of citrate was determined using the Warburg apparatus. Care was taken that only animals free from kidney and urethral concretions were used.

The kidneys from rats fed the rachitogenic diet had slightly lower rates of citrate oxidation. However, regardless of whether the diet was rachitogenic or non-rachitogenic, the kidneys of rats receiving dietary vitamin D had a 24 per cent decrease in the rate of citrate oxidation, a highly significant difference. To see if this was a specific effect on citric acid, the experiment was repeated using other Krebs cycle intermediates as substrates. The oxidation of α-ketoglutarate, succinate and fumarate was unaffected by vitamin D. When pyruvate was used as substrate, there was also no change in rate of oxidation but with a mixture of pyruvate and oxalacetate, which would be expected to yield citrate, dietary vitamin D produced a significant decrease in oxidation. Under these circumstances, a greater amount of citrate accumulated in the flasks with tissue from vitamin D-treated rats. These results were interpreted as showing that vitamin D diminishes the oxidation of citrate to α-ketoglutarate.

Similar experiments with liver homogenates showed no effect of vitamin D on citrate oxidation, in agreement with a previous finding that this vitamin does not increase the citrate content of liver tissue (Steenbock and Bellin, *J. Biol. Chem.* **205,** *985* (*1952*)).

In an attempt to discover how vitamin D affects the oxidation of citrate by kidneys, the addition of the vitamin *in vitro* to homogenates was investigated. No effect was found. Since it is known that vitamin D

increases serum calcium, the authors thought that perhaps ionic calcium added to the kidney homogenates would give a clue. When calcium, as $CaCl_2$, was added in very small concentrations (10^{-4} to 10^{-5} molar), a marked reduction in rate of citrate oxidation occurred. Analyses of kidney homogenates for calcium revealed that the content was slightly elevated by feeding vitamin D. When twice these naturally occurring amounts of calcium were added to "non-vitamin D" homogenates, the reduction of citrate oxidation was not as great as that in homogenates from rats fed vitamin D. Furthermore the addition of Versene, which ties up, or binds, calcium to the reaction mixtures, did not reduce the effect of the dietary vitamin D, but did reduce the slight inhibitory effect of added calcium. It was concluded that the inhibitory effect produced by feeding vitamin D was not due to the increased concentration of calcium.

It was shown earlier by P. G. Tulpule and V. N. Patwardhan (*Biochem. J.* **58,** *61 (1954); Nutrition Reviews* **13,** *61 (1955)*) that the oxidation of pyruvate was almost completely abolished in cartilage slices from rats fed a rachitogenic diet (with no vitamin D) for 20 days. When these results are considered in the light of the above studies of deLuca and associates, it is apparent that vitamin D exerts different effects on different tissues. These two studies illustrate some of the difficulties encountered in attempting to arrive at a true understanding of the metabolic role of many nutrients, particularly in the field of fat-soluble vitamins.

The experiments of deLuca and associates may give an explanation of one function of vitamin D. However, further work is needed to elucidate specifically the effect on citrate oxidation. Just how the effect of vitamin D on citrate metabolism is related to its effect on bone calcification remains the primary problem in this area of nutrition.

NOTES

Dr. Willis A. Gortner, on leave from the Pineapple Research Institute, has accepted a short-term appointment to work with the Committee on Fats of the Food and Nutrition Board of the National Research Council. He will assist the Committee, of which Dr. Paul Day is chairman, in preparing a concise review of the literature and current findings on fats in nutrition.

Letter to the Editor

Dear Sir,

The lack of a general relationship between cancer and nutrition in man despite considerable animal experimentation is emphasised in the article by H. Rusch (*Nutrition Reviews* **14,** *353 (1956)*). It is also pointed out that the general body of evidence suggests that in Africa poor diets are probably the main cause of the high incidence of primary liver cancer in man. This belief is widely held and the significance of diet in causing or altering the production of liver tumors in experimental animals is unquestioned. It would appear, however, advisable to review its application to man, especially since the correlation between malnutrition and this neoplasm is by no means satisfactory.

Primary liver cancer in South Africa arises almost entirely in livers with a severe cirrhosis which undergoes malignant change in about 50% of cases. The cause of this cirrhosis is uncertain, but histologically it would appear of the post-necrotic type. There is no support for the view that it is the direct result of either infantile or adult malnutrition (J. Higginson, B. G. Grobbelaar and A. R. P. Walker, *Am. J. Path.* **33,** *29 (1957)*). While kwashiorkor is almost invariably found in communities where liver

cancer is common, the converse is not true, and liver cancer is rare in Jamaica (G. Bras, S. E. H. Brooks and E. E. Depass *Docum. med. geogr. trop.* **7,** *146* (*1955*)) and in India (V. R. Khanolkar, *Acta Unio int. Cancer* **7,** (*1957*)) although in both areas infantile and adult malnutrition are widespread. The unequivocal demonstration of a severe cirrhosis arising from the fatty liver of kwashiorkor has yet to be made.

There is also no evidence that the widespread undernutrition seen in Central Europe after the recent World Wars has been followed by a significant increase in primary liver cancer. In Malay, Marsden (*Schweiz. Z. Path.* **18,** *644* (*1955*)) states that liver cancer is more common in the Chinese section than in the Malayans although the latter are if anything worse fed. A recent report indicates that this high frequency in Malayan Chinese is confined to first generation immigrants (K. Shammugaratnam, *Brit. J. Cancer.* **10,** *232* (*1946*)).

These observations would indicate that neither infantile nor adult malnutrition alone are sufficient to induce cancer in the liver, and the existence of additional carcinogenic stimuli acting as promoting agents must be accepted.

In man it is advisable to maintain a distinction between malnutrition in infancy and malnutrition in adults, as little is known regarding compensating mechanism in metabolism induced by long continued dietary deficiency. Accepting the Malayan observations on Chinese immigrants, and reports indicating an increased sensitivity of the African liver to hepato-toxic viruses (G. Charmot, R. Camain, and P. Giudicelli, *Bull Soc. path. exot.* **46,** *847* (*1953*); C. Bergeret and F. Roulet, *Acta Tropica.* **4,** *210* (*1947*)) it would appear that the role of diet, if of significance in hepatic carcinogenesis probably lies in dietary liver damage in infancy predisposing the liver in later life to promotive carcinogenic stimuli. The significance of adult malnutrition is as yet uncertain.

I would therefore support the view that not only is more information required regarding the long term effects of malnutrition in infancy, as a possible initiating factor, but also that the search for promoting agents such as viruses, native drugs and medicines should be pursued further before we can ascribe human liver cancer mainly to dietary causes.

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Total Exchangeable Sodium in Hypertension

The efficacy of sodium restriction in hypertension has been examined extensively (*Nutrition Reviews* **11,** *196* (*1953*); **13,** *79* (*1955*)). It has been suggested by several investigators that hypertensive animals and hypertensive patients have a higher tissue content of sodium. In order to study this problem further, E. J. Ross (*Clin. Sci.* **15,** *81* (*1953*)) estimated the total exchangeable sodium (Na_e), total body water, and extracellular fluid in a group of hypertensive individuals.

The patients studied had severe hypertension without congestive heart failure. The total body water was normal. The Na_e was high in ten of 29 patients, the highest value noted being 70 mEq per kilogram of body weight. The extracellular volume, as measured by thiosulfate, was expanded about 10 per cent. Most of the excess sodium, therefore, lay outside the thiosulfate compartment. This sodium averaged 43 mEq per liter of intracellular water in a control group compared to the 94 mEq per liter of the hypertensive group. This extra sodium cannot, of course, be anatomically placed since bone, cells, and compartments outside of the thiosulfate space, although still extracellular, may contain varying amounts of sodium.

An attempt was made to correlate Na_e

with the severity of the hypertensive retinal changes. In general, the more severe the retinopathy the greater was the Na_e. There was a large scatter among the groups, however.

It was not possible from these observations to predict which hypertensive patients would respond to various therapeutic regimens. Neither can a high Na_e be implicated as an etiologic factor in hypertension, since this finding may result from some process related to hypertensive disease. Studies of this type are important though, as they may lead to the differentiation of several types of essential hypertenson with subsequent therapeutic formulations.

Recent Nutrition Books

Nutrition in Japan. 1956. (in English). Published by Nutrition Section, Bureau of Public Sanitation, Ministry of Health and Welfare, Japan, 1956. Pp. 30.

Aspects of Health, Sanitation and Nutritional Status in a Siamese Rice Village. Studies in Bang Chan, 1952–1954. Hazel M. Hauck, with the assistance of Anusith Rajatasilpin Sapha Indrasud, Chumlong Kittivija and Saovanee Sudsaneh. Cornell Thailand Project Interim Reports Series Number Two. Southeast Asia Program, Department of Far Eastern Studies, Cornell University, Ithaca, New York, September 1956. Price $1.00.

Erratum

On page 85 of the March 1957 issue, in the review entitled "A New Growth Factor", the new growth factor discussed is incorrectly described as a gamma lactone. The compound is a delta lactone.

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